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(19)

(54) REMOVING SPECIFIC FACTORS FROM BLOOD

(71) We, MITSUI TOATSU CHEMICALS, INCORPORATED, a Japanese Company, of No. 2—5, Kasumigaseki 3-chome, Chiyoda-ku, Tokyo, Japan, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates *inter alia* to an apparatus for continuous removal of specific substances in blood. More particularly, but not exclusively, the present invention relates to an apparatus comprising an inlet tube, a filter, an adsorber and an outlet tube, the apparatus being arranged for selective removal of the specific substances in blood circulating from the body, through the apparatus and back to the body.

In recent years, the remarkable progress in immunology has brought forth detailed information on various autoantibodies in autoimmune diseases. For example, the antinuclear factor (ANF), especially anti-DNA antibody in systemic lupus erythematosus (SLE) patients and the rheumatoid factor (RF) in rheumatoid arthritis (RA) patients have been suggested to have etiological roles.

Dr. D. S. Terman *et al* (U.S.A.) reported the selective removal of anti-DNA antibody by immunological adsorbents in "Clinical and Experimental Immunology" (1976) *24*, 231—237. In their investigation, they used DNA-cellulose embedded in agar as the adsorbent. The blood prepared for experiments *in vitro* was serum from SLE patients and that for experiments *in vivo* was the whole blood from rabbits immunized with DNA and methylated bovine serum albumin mixed with Freund's complete adjuvant. The blood was passed through a column with a quantitative pump and sampled at a constant interval for analysis of anti-DNA antibody.

They concluded that the DNA-cellulose-agar adsorbent used was effective to selectively remove circulating anti-DNA antibody in both *in vitro* and *in vivo* experiments and that the adsorbent could be expected to be an effective therapeutic agent in clinical medicine. It was also stated that the experimental period of this investigation was rather short and further research should be continued with a view to achieving better results.

Under such circumstances, provision of a new apparatus capable of continuously removing specific factors from blood as contemplated in the present invention contributes considerably to the treatment of the relevant diseases and to elucidation of the causes of such diseases. We believe effective compact apparatus which can continuously separate and treat plasma in large quantities *in vivo* with a wide range of utility has not been previously developed. Artificial kidney machines operating on the whole blood method can involve undesirable flow of adsorbent into the body and destruction of the blood cells. If an appropriate coating is applied to the adsorber to prevent such difficulties, the efficiency is liable to be reduced.

The present invention is the result of extensive research undertaken to develop a satisfactory apparatus. We have found for example that removal of specific factors from blood with remarkably improved efficiency can be achieved by providing a filter before an adsorber in an apparatus wherein the plasma in blood circulating externally of the body is separated by the filter from the blood cells comprising red blood cells, white blood cells and platelets and is treated in the adsorber with adsorbent comprising insolubilised substances such as enzymes, antigens and antibodies which adsorb the specific factors from the plasma.

The invention provides from one aspect an apparatus adapted for use in continuously acting, externally of the body, on blood circulating from the body to the apparatus and back to the body, the apparatus comprising (a) means for the continuous separation of plasma from blood cells which comprise red blood cells, white blood cells and platelets; (b) means operable to remove specific factors from the separated plasma, the said specific factors being selected from anti-DNA antibodies, rheumatoid factor, autoantibodies, antiinsulin antibody, reagins and human im-

munoglobulins for clinical purposes; (c) means for re-mixing the separated blood cells and the plasma from which the said selected specific factors have been removed; (d) an inlet for entry of the blood into the separating means; and (e) an outlet for return of the re-mixed blood to the body.

The removing means preferably comprises an adsorber.

From another aspect, the invention provides an apparatus adapted for use in continuously acting, externally of the body, on blood circulating frm the body to the apparatus and back to the body, the apparatus comprising (a) separating means for continuously filtering plasma from blood cells which comprise red blood cells, white blood cells and platelets; (b) an adsorber containing insolubilized substances selected from enzymes, antigens and antibodies which adsorb and remove from the plasma specific factors selected from anti-DNA antibodies, rheumatoid factor, autoantibodies, anti-insulin antibody, reagins and human immunoglobulins for clinical purposes; (c) means for re-mixing the separated blood cells and the plasma from which the spceific factors have been removed; (d) an inlet for entry of the blood into the separating means; and (e) an outlet for return of the re-mixed blood to the body.

In a preferred embodiment, the apparatus comprises (a) an inlet for the recirculating blood; (b) separating means connected to the inlet and having a single filter membrane therein for separating the plasma from said recirculating blood; (c) an adsorber connected to the filter means and having therein an adsorbent selected from enzymes. antigens and antibodies and in the insolubilized state which is operable to remove from the separated plasma specific factors selected from anti-DNA antibodies, rheumatoid factor, autoantibodies, anti-insulin antibody, reagins and human immunoglobulins for clinical purposes; (d) a pump downstream of the adsorber for exerting a negative pressure on the filter means to aid in the separation of the plasma; (e) means for remixing the plasma from which said specific factors have been removed with the blood from which said plasma has been separated; and (f) an outlet for said recirculating remixed blood.

The human immunoglobulin collectable for clinical purposes in a large quantity from blood, can be human anti-D immunoglobulin administered to Rh(−) women when they have their second children or human anti-tetanus immmoglobulin. The apparatus can also be used for collection of pharmacologically useful substances from non-human animal blood.

The invention also provides a method of obtaining pharmacalogically useful substances selected from anti-DNA antibodies, rheumatoid factor, autoantibodies, anti-insulin antibody, reagins and human immunoglobulins for clinical purposes from blood circulating from the body externally of the body and back to the body, comprising the steps of (a) separating, externally of the body, plasma from blood cells which comprise red blood cells, white blood cells and platelets; (b) procuring the pharmacologically useful substances from the separated plasma; (c) then remixing with the separated blood cells the plasma from which the pharmacologcially useful substances have been procured; and (d) returning the remixed blood to the body.

There now follows a description, to be read with reference to the accompanying drawings, of apparatus embodying the invention. This description, which is also illustrative of method aspects of the invention, is given by way of example only, and not by way of limitation of the invention.

In the accompanying drawings:—

Figure 1 shows a diagrammatic view of the apparatus embodying the invention; and

Figure 2 is a graph illustrating a radio-allergosorbent test.

The apparatus embodying the invention is for continuous removal of specific substances from circulatinng blood. The apparatus comprises an input tube which leads blood from the body to the apparatus; a filter connected to the input tube and capable of continuously separating plasma from blood cells comprising red blood cells, white blood cells and platelets; an output tube connected to an outlet for the blood cells in the filter and arranged to lead blood back to the body; an adsorber containing insolubilised substances such as enzymes, antigens and antibodies for adsorbing specific factors in the plasma and connected to the outlet for the plasma in the filter through a conduit tube; and a conduit tube which leads the plasma from the adsorber into the output tube. To fully realise the function of the apparatus, various auxiliary devices may be developed and used. In some cases, the use of such devices is desirable. Such devices include, for example, a heparin injector for preventing blood from coagulation, a blood pump for flowing plasma at a constant flow rate, a thermostat for maintaining the filter and the adsorbent at a constant temperature, a device for removing bubbles and a monitoring unit for checking whether the apparatus is operated without trouble or not.

The function of the present apparatus will now be explained in further detail with reference to Fig. 1.

In Fig. 1, blood taken out from the artery of the body through an input tube 1 is brought to a plasma filter 3 at a constant rate by means of a blood pump 2. The filter comprising a single filter membrane 3—1, a channel 3—2 and a membrane support 3—3 separates plasma from blood cells, the latter

being recirculated back to the body through an outlet tube 7. While the plasma separated in the filter is led to an adsorber 5 through a conduit tube; heparin is added through a heparin injector 4 to prevent coagulation. The plasma from which specific factors have been removed in the adsorber 5 is pumped by a filter pump 6, mixed with the blood cells and recirculated back to the body through the outlet tube 7.

Useful as the input tube 1, the conduit tube and the output tube 7 are silicone tubes and soft polyvinyl chloride tubes. A small roller pump is suitable as the blood pump 2. Useful as the filter 3 are a filter of the Kiil type and a filter of hollow fiber type used for hemodialysis provided with a membrane having an effective area of less than 1 m^2 depending upon the desired purpose. We believe a filter membrane with a pore size ranging from 0.15 μm to 0.8 μm is optimal to separate the blood cells and the plasma. The membranes may be those made of cellulose acetate, polycarbonate, polyvinyl chloride, epoxy resin fibers, glass fibers, polyamide, regenerated cellulose, cellulose nitrate, "Teflon" (Registered Trade Mark), nylon, cellulose or the like. The membranes are sterilized before actual use. A Kiil type filter is a dialysis filter which includes two separate, parallel-disposed filtration cells each furnished with a dialysis membrane. The membranes extend, parallel to one another, transversely of their respective cells separating each into two parts. Regenerated cellulose such as Cellophane (Registered Trade Mark) can form the membranes. Whole blood is admitted simultaneously to one part of each of the two cells and is caused to flow along one side of each membrane through which plasma can pass. Plasma collects and is recoverable from the cell parts to the opposite side of the respective membranes, blood cells and platelets being left behind and recoverable from the said one part of the filtration cells.

The adsorber 5 is generally a column packed with an insolubilized substance. The insoluble supports must be resistant to serum enzymes, free from non-specific adsorption and stable at a temperature between 0°C and 40°C. Preferred examples of the insoluble supports include cross-linked dextran, cross-linked polyacrylamide, polyaminopolystyrene, cellulose, cellulose derivatives, agarose and polymerized proteins. These insoluble supports are generally modified according to the specific circulating factor to be removed. For instance, DNA-Sepharose prepared by covalently bonding DNA to Sepharose 4B (Pharmacia; agarose) is a suitable insolubilized substance to bind anti-DNA antibody specifically. "Sepharose" is a Registered Trade Mark.

The filter pump 6, which is of the same type as the blood pump 2, facilitates filtration by exerting negative pressure to the filter 3.

A result of animal experiments carried out using the present apparatus will be described hereunder.

Example 1.

Adsorption of anti-egg albumin antibody *in vivo*.

A male rabbit with a body weight of 3.3 Kg was used in this experiment. The rabbit was immunized by intradermic injection of 8 mg of egg albumin mixed with Freund's complete adjuvant to the back. After the lapse of 7 weeks a perfusion cannula and a recirculation cannula were inserted into the arteries *carotis communis* at a distance of 5 mm, which were then connected to the apparatus shown in Fig. 1 to circulate the blood *in vitro*. The filter membrane in the filter was a cellulose membrane having an effective area of 43 cm^2 and pores of 0.2 μm in diameter. The adsorbent was a column packed with 13 ml of egg albumin-Sepharose 4B. The pumps were controlled so as to adjust the flow rate of the whole blood to 7 ml/min and that of the plasma to 1.6 ml/min. After the lapse of 90 minutes, the egg albumin-Sepharose 4B column was exchanged and the treatment was continued for further 90 minutes before all the perfused blood was returned to the body. During this treatment of plasma, 1000 units/Kg of heparin was added to prevent coagulation. The egg albumin-Sepharose 4B to which the antibody had been adsorbed was washed with 100 ml of a phosphate buffer kept at pH 7.5. The adsorbed antibody was then dissociated with 20 ml of a 0.1M alanine buffer kept at pH 2.3. The dissociated antibody was neutralised with a 1.0M alanine buffer kept at pH 10.0, dialyzed against distilled water for 3 days and lyophilized whereby 480 mg of a powder was obtained. The result of electrophoresis showed that the powder was composed predominantly of γ-globulin with a small amount of α-globulin contaminant. The amount of antibody in the powder was determined as 450 mg by a radioallergosorbent test (RAST) using the purified anti-egg alubumin antibody as standard. Two guinea pigs were intradermally administered 0.1 ml aliquots of 5, 1 and 0.2 μg/ml solutions of this antibody. After 4 hours, the guinea pigs were injected intravenously with 0.5 ml of 10% Evan's blue and 0.5 ml of 1% egg albumin. After the lapse of 30 minutes, the result of PCA (passive cutaneous anaphylaxis) reaction was measured, which is shown in Table 1.

TABLE 1

	Concentration of the antibody obtained from egg albumin-Sepharose 4B (μg/ml)		
	5	1	0.2
Diameter of the reaction plaque (mm)	13.8	7.9	2.5

The antigen values in the blood measured by radioallergosorbent testing are shown in Fig. 2. Consequently, the amount of the antibody which has been removed from plasma by the present apparatus is calculated as $3.3 \times 40 \times (4.0 - 0.9) = 410$ (mg), provided that the amount of the plasma is 40 ml/kg. The calculated value is almost equal to 450 mg that is the amount of the antibody adsorbed on egg albumin-Sepharose 4B in the present apparatus.

Example 2.
Effects of hematological image and blood biochemistry.

A male dog having a body weight of 14 kg was used for this experiment. A perfusion cannula was inserted into the femoral artery and a circulation cannula into the femoral vein, both of which were connected to the apparatus shown in Fig. 1 to circulate blood *in vitro*. A filter membrane used in the filter was a cellulose membrane having an effective area of 300 cm^2 and a pore diameter of 0.65 μm. The blood was obtained from the femoral artery at a flow rate of 150 ml/min. under a pressure of 50 mmHg. The plasma was separated in the filter at a flow rate of 5 ml/min. The plasma was mixed with the blood cells near the outlet of the filter and circulated back to the body through the femoral vein. After perfusion for 90 minutes, the effects on hematological image and blood biochemistry were investigated. The change in hematological image is shown in Table 2 and that in blood biochemistry in Table 3. The treatment had little effect on the two properties. An electrocardiogram was also studied and shown to be unaffected by the treatment.

TABLE 2

	Before	After circulation (hr)		
		0	2	24
Ht (%)	40.4	39.5	39.0	39.7
Hb (g/dl)	14.3	13.6	13.3	13.8
RBC ($10^4 \times mm^3$)	624	601	596	586
WBC ($10^2 \times mm^3$)	96	87	138	158
Platelet ($10^4 \times mm^3$)	38	38	36	37

TABLE 3

Blood chemistry findings

	T.P. (g/dl)	Albumin (g/dl)	Total cholesterol (mg/dl)	Urea-N (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Before	7.3	3 3	268	26.5	0.50	0.40
After	6.9	3.1	270	27.0	0.48	0.35

WHAT WE CLAIM IS:—

1. An apparatus adapted for use in continuously acting, externally of the body, on blood circulating from the body to the apparatus and back to the body, the apparatus comprising (a) means for the continuous separation of plasma from blood cells which comprise red blood cells, white blood cells and platelets; (b) means operable to remove specific factors from the separated plasma, the said specific factors being selected from anti-DNA antibodies, rheumatoid factor, autoantibodies, anti-insulin antibody, reagins and human immunoglobulins for clinical purposes; (c) means for re-mixing the separated blood cells and the plasma from which the said selected specific factors have been removed; (d) an inlet for entry of the blood into the separating means; and (e) an outlet for return of the re-mixed blood to the body.

2. An apparatus according to claim 1, wherein the removing means comprises an adsorber.

3. An apparatus adapted for use in continuously acting, externally of the body, on blood circulating from the body to the apparatus and back to the body, the apparatus comprising (a) separating means for continuously filtering plasma from blood cells which comprise red blood cells, white blood cells and platelets; (b) an adsorber containing insolubilized substances selected from enzymes, antigens and antibodies which adsorb and remove from the plasma specific factors selected from anti-DNA antibodies, rheumatoid factor, autoantibodies, anti-insulin antibody, reagins and human immunoglobulins for clinical purposes; (c) for re-mixing the separated blood cells and the plasma from which the specific factors have been removed; (d) an inlet for entry of the blood into the separating means; and (e) an outlet for return of the re-mixed blood to the body.

4. Apparatus for continuously separating from a stream of recirculating blood the plasma component thereof and removing specific factors from said plasma, which apparatus comprises: (a) an inlet for the recirculating blood; (b) separating means connected to the inlet and having a single filter membrane therein for separating the plasma from said recirculating blood; (c) an adsorber connected to the filter means and having therein an adsorbent selected from enzymes, antigens and antibodies and in the insolubilized state which is operable to remove from the separated plasma specific factors selected from anti-DNA antibodies, rheumatoid factor, autoantibodies, anti-insulin antibody, reagins and human immunoglobulins for clinical purposes; (d) a pump downstream of the adsorber for exerting a negative pressure on the filter means to aid in the separation of the plasma; (e) means for remixing the plasma from which said specific factors have been removed with the blood from which said plasma has been separated; and (f) an outlet for said recirculating remixed blood.

5. An apparatus according to any one of the preceding claims wherein the separating means comprises a filter membrane having a pore size ranging from 0.15 μm to 0.8 μm.

6. An apparatus according to any one of claims 1 to 5, wherein the separating means comprises a filter membrane having an effective area less than 1 m^2.

7. An apparatus according to any one of the preceding claims including a blood pump arranged to effect blood flow to the means which separate the plasma from the blood cells.

8. An apparatus according to any one of claims 1 to 3, or any claim dependent on claim 1, 2 or 3, further including a pump arranged to effect passage of the plasma through the means which separate the said selected specific factors from the plasma.

9. An apparatus according to any one of the preceding claims, further including a heparin injector to prevent coagulation.

10. Apparatus adapted for use in acting externally of the body, on blood circulating

from the body to the apparatus and back to the body, constructed, arranged and adapted to operate substantially as hereinbefore described with reference to Figure 1 of the accompanying drawings.

11. A method of obtaining pharmacologically useful substances selected from anti-DNA antibodies, rheumatoid factor, autoantibodies, anti-insulin antibody, reagins and human immunoglobulins for clinical purposes from blood circulating from the body externally of the body and back to the body, comprising the steps of (a) separating, externally of the body, plasma from blood cells which comprise red blood cells, white blood cells and platelets; (b) procuring the pharmacologically useful substances from the separated plasma; (c) then re-mixing with the separated blood cells the plasma from which the pharmacologically useful substances have been procured; and (d) returning the re-mixed blood to the body.

12. A method according to claim 11, wherein said procuring step is carried out by adsorption from the plasma.

13. A method according to claim 12, wherein the procuring step is carried out by insolubilized substances selected from enzymes, antigens and antibodies which adsorb the pharmacologically useful substances from the plasma; the separation step being carried out by filtration.

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1562546 COMPLETE SPECIFICATION

3 SHEETS *This drawing is a reproduction of the Original on a reduced scale*

Sheet 1

FIG. 1

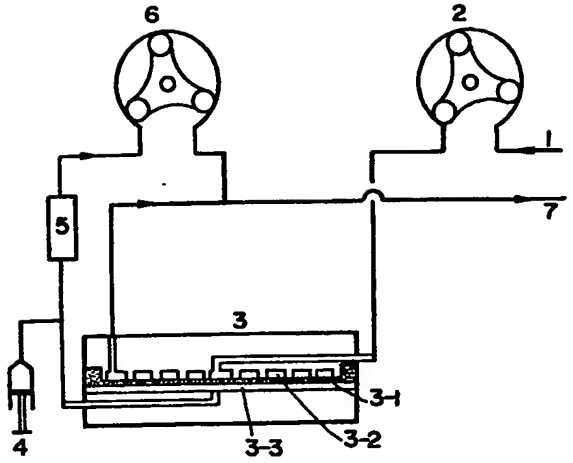

FIG. 2

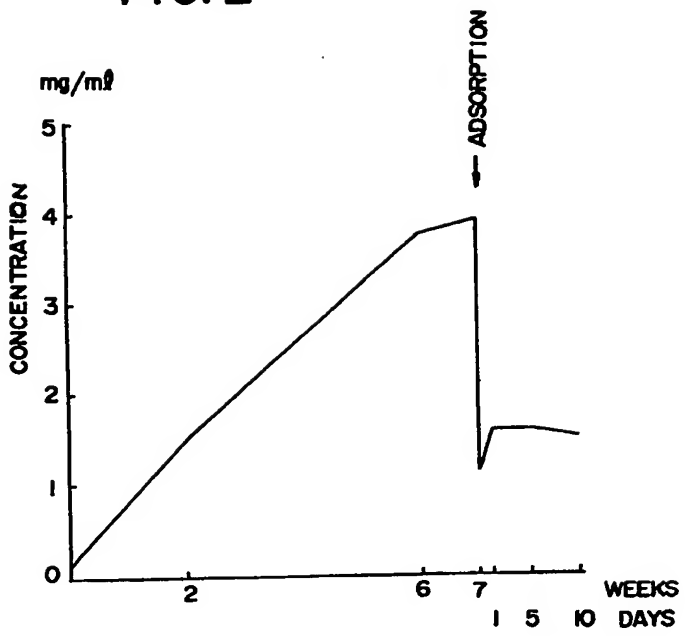

FIG. 3